

be able to produce the recombinant plasmid even with the disclosure in the original Fig. 2. In previous amending of Fig. 2, the applicant disclosed further that the pT7T3 18U vector was used in producing the recombinant plasmid only to clarify the process of producing. The pT7T3 18 U vector is a well-known vector which is being used widely as cloning cDNA.

In response to rejecting claim 6 as being indefinite as to “A biological verification method for a recombinant human *lactoferrin*”, the applicant amended claim 6 such that claim 6 claims the method to verify the biological activity of the recombinant human *lactoferrin*. Verifying the recombinant lactoferrin as the human lactoferrin includes three stages of transcription, translation, and effect. The invention claims the method of verifying the effect, the 3rd stage. As it were, the transcription is verified by RT-PCR and the translation is verified by using the antibody to the human lactoferrin from the culture or insect cell, which are prior arts, the invention claims the method to verify the biological activity of the human lactoferrin by measuring the anti-bacterial activity after mixing with the pathogenic microorganisms.

In response to rejecting claim 6 under 35 U.S.C. 103(a) as being unpatentable over Naidu *et al.*, we deleted *Salmonella typhimurium* from the microorganisms.

CONCLUSION

The applicant believes that the rejections were obviated by the amendment of specification, claims, and drawings, and the application is now in condition for allowance: therefore, reexamination, reconsideration and allowance of the claims are respectively requested. If there is any additional comments or requirements from the examination, the applicant asks for a non-final office action.

The Commissioner is hereby authorized to charge payment of any additional fees associated with this communication, or credit any over-payment to Deposit Account No. 16-0310.

Very truly yours,

Park & Sutton LLP

Dated: 2/9, 2005

By 

John K. Park

Regis. No. 37,904

3255 Wilshire Blvd., Suite 1110

Los Angeles, California 90010

Tel: (213) 389-3777

Claim Amendment under 37 CFR 1.121(c)

1. (Currently Amended) A method for producing human *lactoferrin* by using an insect cell comprising the steps of:
 - (a) combining a transfer vector pBacPAK8 with a recombinant plasmid ~~pHLf-8~~ (that is the same as pT7T3-hLf which has pT7T3-18U backbone, pharmacia and human lactoferrin gene using SalI and HindIII site) which has a 2.1 kb full gene including the start codon and the signal sequence of human lactoferrin using SalI and HindIII site to produce a recombinant expression vector pBacLf ~~(that is the same as pBacPAK-hLf)~~ modified to permit the regulation of a *lactoferrin* gene by a polyhedrin promoter in a vector pBacPAK8;
 - (b) cotransfecting said recombinant expression vector together with a help vector BacPAK6 viral DNA into an insect cell Sf9 in a culture medium to produce a recombinant insect cell Sf-Lf, and producing a recombinant insect virus from said recombinant insect cell resulting from homologous recombination ~~through AcMNPV and AcNPV site~~ that AcMNPV and AcNPV site of said recombinant expression vector cut in said help vector; and
 - (c) producing human *lactoferrin* using said recombinant insect virus.

2. (Original) The method of claim 1, wherein said producing a recombinant insect virus step further comprises the step of performing a centrifugal separation of the culture medium containing the recombinant insect cell cultured in the producing step (b) to obtain a progeny virus from the insect cell contained in the upper layer.

3. (Cancelled)

4. (Currently Amended) A recombinant insect virus produced by a method comprising the steps of:
 - (a) combining a transfer vector pBacPAK8 with a recombinant plasmid ~~pHLf-8~~ (that is the same as pT7T3-hLf which has pT7T3-18U backbone, pharmacia and human lactoferrin gene using SalI and HindIII site) which has a 2.1 kb full gene including the start codon and the signal sequence of human lactoferrin using SalI

and HindIII site to produce a recombinant expression vector ~~pBacLf (that is the same as pBacPAK-hLf)~~ pBacPAK8 modified to permit the regulation of a *lactoferrin* gene by a polyhedrin promoter in a vector pBacPAK8;

(b) cotransfecting said recombinant expression vector together with a helper vector BacPAK6 viral DNA into an insect cell Sf9 in a culture medium to produce and culture a recombinant insect cell Sf-Lf; and

(c) producing a recombinant insect virus from said recombinant insect cell Sf-Lf resulting from homologous recombination ~~through AcMNPV and AcNPV site that~~ AcMNPV and AcNPV site of said recombinant expression vector cut in said helper vector.

5. (Cancelled)
6. (Currently Amended) A biological verification method for a recombinant human *lactoferrin*, comprising the steps of:
 - (a) mixing human *lactoferrin* produced by the method of claim 1 with a pathogenic microorganism selected from the group consisting of *Pseudomonas cepacia*, *Pseudomonas putida*, ~~*Salmonella typhimurium*~~, *Pseudomonas fluorescence* and *E. coli* 300; and,
 - (b) measuring anti-bacterial activity of said mixture against the pathogenic microorganism.

SPECIFICATION AMENDMENT UNDER 37 CFR 1.121 (B) (1)

Please replace the paragraph on lines 18-26, page 5 of the original specification with the following replacement paragraph.

5

The novel method for producing human *lactoferrin* by using an insect cell comprises the following steps: (a) combining a transfer vector 1 with a recombinant plasmid ~~pHLf 8 2~~ to prepare a recombinant expression vector ~~pBacLf 3~~ pBacPAK8 with a recombinant plasmid which has a 2.1 kb full gene including the start codon and the signal sequence of human lactoferrin using Sall and HindIII site to produce a recombinant
 10 expression vector pBacPAK-hLf modified to permit the regulation of a *lactoferrin* gene by a polyhedrin promoter in a vector pBacPAK8; (b) cotransfecting the said recombinant expression vector together with a help vector ~~pBacPAK6 4~~ BacPAK6 viral DNA into an insect cell Sf9 ~~[[5]]~~ in a culture medium to ~~prepare~~ produce a recombinant insect cell Sf-
 15 Lf, and producing a recombinant insect virus from the said recombinant insect cell resulting from homologous recombination that AcMNPV and AcNPV site of said recombinant expression vector cut in said help vector; and (c) producing human *lactoferrin* ~~from the recombinant insect cell Sf Lf 6~~ using said recombinant insect virus.

20

Please replace the paragraph on lines 4-9, page 6 of the original specification with the following replacement paragraph.

To prepare the recombinant insect virus, the transfer vector 1 is first combined
 25 with the recombinant plasmid 2 to produce the recombinant expression vector ~~pBacLf~~ (pBacPAK-hLf) 3 modified to permit the regulation of a *lactoferrin* gene by a polyhedrin

promoter in a vector ~~pBacPAK~~ pBacPAK8, and the recombinant expression vector is cotransfected with the help vector BacPAK6 4 into the insect cell Sf9 5 in a culture medium to produce the recombinant insect cell Sf-Lf 6, from which the recombinant insect virus is produced.

5

Please replace the paragraph on line 26 page 7 – line 1 page 8 of the original specification with the following replacement paragraph.

10 Fig. 2 is a flow chart showing a process of producing a recombinant expression vector ~~pBacLf~~ pBacPAK-hLf according to the present invention.

Please replace the paragraph on lines 2 – 4, page 8 of the original specification
15 with the following replacement paragraph.

Fig. 3 is a photograph showing the electrophoresis pattern of the recombinant expression vector ~~pBacLf~~ pBacPAK-hLf after cleavage with a restriction enzyme according to the present invention.

20

Please replace the paragraph on lines 14 – 15, page 8 of the original specification with the following replacement paragraph.

EXAMPLE 1: Cultivation of Insect Cell and Production of Recombinant Expression Vector pBacLf pBacPAK-hLf

Please replace the paragraph on line 24 page 8 – 7 page 9 of the original specification with the following replacement paragraph.

To transfer a *lactoferrin* gene into a baculovirus gene, a 2.1 kb full gene including the start codon and the signal sequence of the *lactoferrin* was produced from the existing recombinant plasmid for cloning a transfer vector (pBacPAK8, Clontech Co.) including the polyhedrin promoter site (5.5 kb), and then inserted into *E. coli* in the same direction of the polyhedrin promoter to prepare a recombinant expression vector. The existing recombinant plasmid has a 2.1 kb full gene including the start codon and the signal sequence of the *lactoferrin* and is produced with the pT7T3 18U vector(Pharmacia Co.) which was commercially available as a cloning cDNA in the present invention. Of course, the recombinant plasmid can be produced with a different kind of vectors. The transfer vector (pBacPAK8) was commercially available from Clontech laboratories Inc. (1020 East Meadow Circle, Palo Alto, C.A. 94303-4230, USA). The recombinant expression vector thus obtained was treated with a restriction enzyme to verify the *lactoferrin* gene, which was named “pBacPAK-hLf”. A process for producing the expression vector pBacPAK-hLf is illustrated in Fig. 2.

Please add the following paragraph right after line 7, page 9 of the original specification.

Fig. 2 is a photograph showing a basic process for producing the recombinant DNA, and to be more particular it is as follow. The human *lactoferrin* gene was amplified to make SalI and HindIII site before and behind of the human *lactoferrin* gene, and the

lactoferrin gene amplified was put between SalI and HindIII of pT7T3 18U vector, and in result the recombinant plasmid was produced and named pT7T3-hLf. So the pT7T3-hLf had pT7T3 18U backbone(Pharmacia) and human lactoferrin gene using SalI and HindIII site. And then the human *lactoferrin* gene was taken out from the pT7T3-hLf by treating with
 5 restriction enzymes Bam HI and HindIII/Klenow, and put between SalI and HindIII of pBacPAK8 vector, and in result the recombinant expression vector was produced and named pBacPAK-hLf.

10 Please replace the paragraph on lines 24 page 9 – 18 page 10 of the original specification with the following replacement paragraph.

Sf9 cells were inoculated in an amount of about 1.0×10^6 cells in a Grace's basic medium containing 10% FBS and were cultured for 4 hours. The insect cell was washed
 15 with the Grace's basic medium twice and remained at the ambient temperature for 30minutes. A mixture of a virus DNA (BacPAK 6, Clontech Co.) and a recombinant transfer vector (pBacPAK-hLf) prepared for liposome-mediated transfection, together with lipofectin was dropped on a cell monolayer. The mixture was added to the Grace's medium containing serum and antibiotics and was cultured at 28 °C for 5 days. The
 20 supertant was diluted with the culture solution in ten stages, 3 to 5 times and was inoculated into the insect cell Sf9 cultured in a monolayer on a 60mm-diameter plate. As AcMNPV and AcNPV site in pBacPAK-hLf are located before and behind the human *lactoferrin* gene and they are homologous with nucleotide sequence in Baculovirus(BacPAK6), homologous recombination occurs if the insect cell Sf9 is
 25 infected with pBacPAK-hLf and BacPAK6. In result a section of pBacPAK-hLf cuts in BacPAK6. When the virus was adsorbed, the dissolved agarose-containing medium was hardened on the insect cell. After 6 to 7 days, the insect cell with the agarose-containing medium was dyed with neutral red, which dyes dead cells distinguished and forms a

plaque. A microscope was used to select a plaque in which the polyhedrin by the infection with the recombinant virus was not formed. The plaque with the agarose was sucked up with a Pasteur pipette and was suspended in a 1ml medium. In order to verify whether the recombinant virus contains the *lactoferrin* gene, another insect cell Sf9
5 cultured in a new Grace's medium was infected with the recombinant virus and the recombinant virus DNA was isolated, after which the electrophoresis patterns of the isolated DNA were compared by agarose gel electrophoresis. By polymerase chain reaction (PCR) using a primer capable of amplifying *lactoferrin*, it was identified and after treatment with a restriction enzyme, the *lactoferrin* gene (2.1 kb) was identified by
10 Southern blot analysis.